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ARTICLE

Quantitative elemental localisation in plants using ion beam microprobe analysis

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ABSTRACT In plant biology there are efforts to study the accurate elemental concentrations and localisations in plant tissues on a microscopic scale. Ion beam analysis successfully introduced into material science, geology, archaeology and aerosol study offers a good possibility to carry out quantitative elemental investigations in plant samples. In this work we show the applicability of ion beam analysis at the microprobe facility available in ATOMKI, Debrecen, in plant studies. Concentrations and distributions of major, micro- and trace elements were determined within the roots of *Bidens tripartitus*. Metals were found in higher concentrations in the rhisodermis than in the inner tissues. Besides concentration values maps of elemental distribution in the tissues are also presented. Ion beam microprobe analysis can successfully complement bulk techniques in studying the uptake and transport processes of various elements in plants.
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KEY WORDS

imaging elemental distribution
ion beam microprobe
PIX
STIM

Knowledge on concentration and localisation of elements is required in many fields of plant studies such as fundamental processes, nutrition, elemental toxicity (Mesjasz-Przybyłowicz and Przybyłowicz 2002). Macronutrients (C, H, O, N, K, Ca, Mg, P and S) are present in plant tissues at concentrations above 0.1% dry weight. Micronutrients (B, Cl, Cu, Fe, Mn, Mo, Ni and Zn) can be found in much smaller concentrations (Asher 1991). Other elements may be essential for some species only, and some elements are clearly toxic. Fundamental physiological processes are affected by mineral nutrients and altered by the uptake of minerals such as chromium (Hörcsik et al. 2007). The distribution of toxic elements in plants indicates possible transport pathways, which can elucidate the adaptation of plants growing in a hostile environment (Lakatos et al. 1999). An exciting application of these adaptations is phytoremediation when contaminated soil is purified using plants that hyperaccumulate certain heavy metals (Lakatos et al. 2001). All of these expanding research areas demonstrate the increasing need for quantitative microanalysis.

Imaging the elemental distribution in biological tissues can be a challenging task. Staining methods usually lack sensitivity to detect trace elements and reagents may compete with the endogenous ligands. Atomic spectroscopic techniques like electron microprobe, ion beam analysis, synchrotron radiation microprobe, secondary ion mass spectrometry and laser ablation inductively coupled mass spectrometry were proposed as spatially resolved analytical techniques for element imaging. All of these methods are valuable complements to bulk analysis, with different strengths and limitations.

Ion beam microprobe analysis comprises various techniques using focused ion beams. Proton induced X-ray emission (PIXE) similarly to electron probe X-ray microanalysis and synchrotron radiation microprobe is based on the detection of characteristic X-rays generated by the rearrangements of electrons after excitation caused by the irradiation and emitted from the sample. Because of the higher mass of protons, PIXE is performed with about a 100-fold sensitivity compared to electron microprobe analysis. Compared to

Table 1. Elemental concentrations (μg/g) in different tissues of *Bidens tripartitus*.

	rhisodermis	parenchyma	vascular cylinder
Na	22800	11100	7500
Mg	1650	600	500
Al	1700	<40	1400
Si	4300	120	1450
P	1100	400	200
S	5350	1950	1150
Cl	11700	9900	15300
K	9900	5500	5600
Ca	6900	2100	2900
Ti	795	47	26
Cr	21	18	7
Mn	74	18	14
Fe	1170	120	70
Ni	23	8	<3
Cu	26	<5	6
Zn	43	20	13

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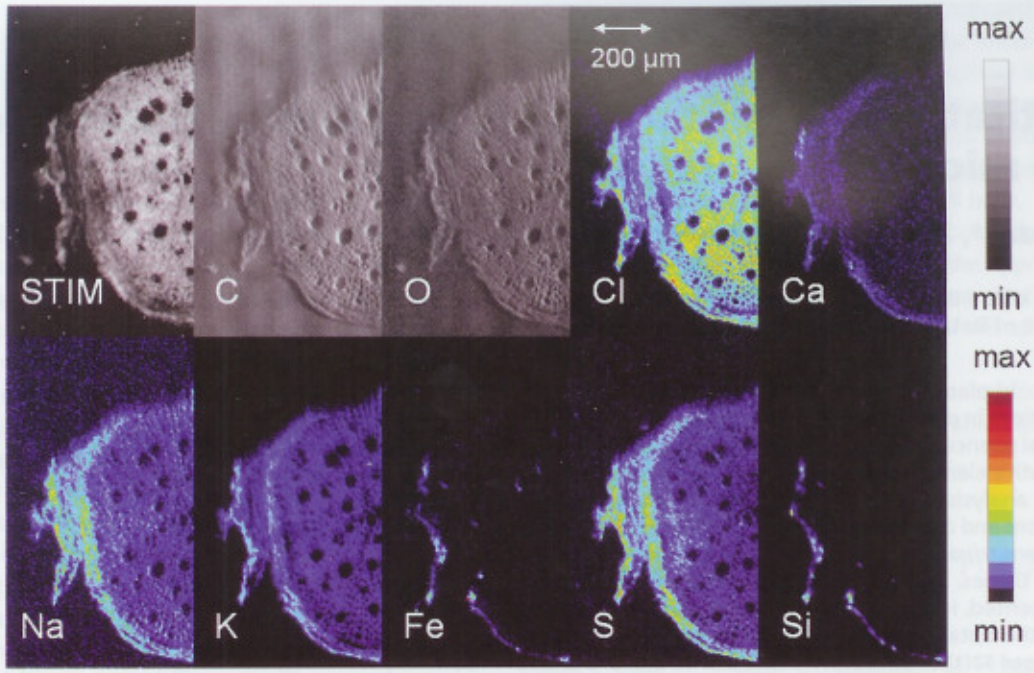

Figure 1. STIM energy loss map and elemental maps of a trifid blur marigold section.

synchrotron radiation X-ray fluorescence (SRXRF), PIXE is less sensitive (typical detection limits in biological matrix are 1-10 ppm for PIXE, and 0.1-1 ppm for SRXRF), but fully quantitative, no standards are required (Lobinski et al. 2006). Additional ion beam analytical techniques such as Rutherford backscattering spectrometry and scanning transmission ion microscopy (STIM) can be used simultaneously with PIXE to characterize the sample mass pixel by pixel if needed.

Materials and Methods

The ion beam microprobe in Debrecen

The microprobe facility is located on the 0° beam line of the 5 MeV Van de Graaff accelerator in the Institute of Nuclear Physics of the Hungarian Academy of Sciences (Kertész 2005). An ultra thin windowed and a conventional Be windowed Gresham Si(Li) X-ray detectors are used to determine the concentrations and distributions of elements in the C to Fe and S to U regions, respectively. The detectors, with 30 mm^2 active area each, are placed at 135° to the incident beam, while the sample is usually perpendicular to the beam. The sample is positioned with a 5-axis motorized goniometer. The accumulated charge irradiating the sample is measured with a Faraday cup in the case of thin samples, such as biological sections. STIM is routinely used to characterize the mass distribution of the samples. Particles lose energy when travelling through the sample. The energy distribution of the incoming particles is measured by an Ortec type "ULTRA" ion-implanted Si detector with excellent energy resolution. The energy loss depends on the density of the sample therefore it gives insight to the local mass. This density distribution also pictures the morphology of the sample. For biological samples, generally a proton beam with 2 MeV is focused to the required resolution which was 2.5 x 2.5 μm^2 in the measurements on plants shown below. Currently the highest achievable resolution is 1 x 1 μm^2 in our laboratory. Concentration values are calculated with the upgraded PIXEKLM program package (Szabó and Borbély-Kiss 1993).

Plant samples

Plants were collected from the wastewater sedimentation pond system of the former leather processing facility in Kunszentmárton (Keresztúri et al. 2006). Trifid bur marigold (*Bidens tripartitus*) roots were quench frozen in liquid nitrogen. 15-20 µm thick sections were cut using a cryo-microtome and the samples were freeze-dried. The specimens were mounted between two layers of pioloform films.

Results

STIM map and selected elemental maps for one specimen are shown in Figure 1. Three regions, rhisodermis, parenchyma and vascular cylinder are clearly distinguishable in the maps due to the differences in density and elemental concentrations. Average concentration values based on three samples are summarized in Table 1.

In the trifid bur marigold samples the vascular cylinder was rich in chlorine, while metals were found in the rhisodermis in higher concentrations than in the inner tissues. The titanium and the iron concentrations were about 800 and 1200 µg/g respectively in the rhisodermis, while chromium, manganese, copper, nickel and zinc were much less abundant. The high titanium and iron concentrations in the rhisodermis but not in the vascular cylinder indicate that these metals mostly remain trapped in the root and do not enter the ion flux upwards in high quantity.

We can conclude that ion beam microprobe analysis at our facility has the potential to contribute to plant research with studies of elemental concentrations and distributions. Information on the localisation of heavy metals provided by the technique may lead to a better understanding of the pathways of these elements which in turn can help clarify the phenomenon of heavy metal resistance.

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